Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 2480–2486
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

A pyran-2-one and four meroterpenoids from *Thapsia transtagana* and their implication in the biosynthesis of transtaganolides

Juan J. Rubal [a], F.J. Moreno-Dorado [a], Francisco M. Guerra [a], Zacarías D. Jorge [a], Abderrahmane Saouf [b], Mohamed Akssira [b], Fouad Mellouki [b], Raúl Romero-Garrido [a], Guillermo M. Massanet [a,*]

[a] *Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Puerto Real, 11510-Cádiz, Spain*
[b] *Faculté des Sciences et Techniques, Université Hassan II Mohammedia, LCBA, UFR C35/97, BP 146 Mohammedia 20650, Morocco*

Received 28 May 2007; received in revised form 15 June 2007
Available online 31 July 2007

Abstract

Four meroterpenoids (**3a**, **3b**, **4** and **5**), a prenylated pyran-2-one (**2**) along with the known compounds 7-*O*-geranylscopoletin (**1**), and thapsitranstagin (**6**) have been isolated from the roots of *Thapsia transtagana*. The presence of **1** and **2** supports the biogenetic hypothesis that transtaganolides, a group of bioactive metabolites, are meroterpenoids which come from an O-prenylated coumarin *via* successive pericyclic reactions.

Keywords: *Thapsia transtagana*; Apiaceae; Meroterpenoids; Transtaganolides; Prenylated coumarin; Guaianolide; Secocoumarin; Thapsianolide; Biogenetic hypothesis

1. Introduction

The genus *Thapsia* is constituted by ten species distributed in the western Mediterranean area and extended to the Atlantic coast of the Iberian Peninsula and Morocco (Pujadas and Roselló, 2003).

Despite its reduced extension, a relatively large number of secondary metabolites have been isolated from the genus *Thapsia*, sesquiterpenoids (Christensen et al., 1997) and phenylpropanoids (Liu et al., 2006), being the more significants. Since the discovery of thapsigargins from *Thapsia garganica* (Rassmussen et al., 1978) the interest for the phytochemical studies on *Thapsia* has been increased. Thapsigargin, the lead compound which names the family and other members of this group of guaianolides are selective and irreversible inhibitors of the ubiquitous sarco-endoplasmatic reticulum Ca^{2+} ATPases (Treiman et al., 1998). Thapsigargin has also been shown to restore apoptotic function in cancer cell lines (Furuya et al., 1994).

In the course of our evaluation of *Thapsia* species as a source of new thapsigargin analogues, we described a new group of secondary metabolites that we named transtaganolides (Saouf et al., 2005). The origin of the very uncommon structure of transtaganolides is quite intriguing. As their structures suggest a terpenoid nature, a biogenetic route starting from geranyl pyrophosphate has been proposed by Appendino et al. (2005), although the same authors also proposed a quite different and more impressive way involving a phenol cleavage and a cascade pericyclic process.[1]

* Corresponding author. Tel.: +34 956 016366; fax: +34 956 16288.
E-mail address: g.martinez@uca.es (G.M. Massanet).

[1] In reference Appendino et al. (2005), the authors reported two new transtaganolides and named them as basiliolides. We consider that the generic name transtaganolides, previously suggested by us after the very first description of these compounds (Saouf et al., 2005) should prevail for all the members of the family for the sake of clarity.

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.06.023

Aside their striking architecture, transtaganolides shows an additional interest as SERCA-inhibitors, as stated by recent reports (Appendino et al., 2005; Navarrete et al., 2006). The above facts prompted us to undertake a new phytochemical study of *T. transtagana*.

As a result of our reinvestigation, together with the compounds previously isolated (Saouf et al., 2005, 2006), new minor metabolites have been identified: the prenylated pyran-2-one **2**, three meroterpenoids belonging to the family of transtaganolides (**3a**, **3b**, and **4**), a lactonic meroterpenoid **5** along with the known compounds 7-*O*-geranylscopoletin **1** (Larsen and Sandberg, 1970), thapsitranstagin **6** (Rasmusssen et al., 1981), and haplonolide **11** (Gunes, 2001). Herein we describe the structural elucidation of **2**, **3a**, **3b**, **4** and **5**.

The presence in *T. transtagana* of compounds **1**, **2**, **3a**, **3b**, **4** and **5** provides evidences about the biosynthetic origin of transtaganolides.

2. Results and discussion

Compound **2** showed in its HREIMS a molecular ion $[M]^+$ at *m*/*z* 362.1752, compatible with the molecular formula, $C_{20}H_{26}O_6$, inferring eight degrees of unsaturation. The characteristic features of the ^1H and ^{13}C NMR spectra together with absorption bands at 1740, 1648 and 1557 cm^{-1} due to the stretching vibrations of the three double bounds (C=O, C5–C6 and C3–C4) in the IR spectrum (Breda et al., 2003) suggested that **2** bears a pyran-2-one ring. The presence of only two doublets at δ_H 6.24 (H-3, 9.5 Hz) and at δ_H 7.25 (H-4, 9.5 Hz) indicated that C-5 and C-6 are substituted.

The ^1H NMR spectra shows two singlets at δ_H 3.30 (2H) and 3.57 (2H), characteristic of two methylene groups located on two alkoxycarbonylmethyl groups. The proton signal at δ_H 3.70 (*s*, 3H) was assigned to a methoxy ester group belonging to the alkoxycarbonylmethyl group attached at carbon C-5 of the pyran-2-one ring, as was determined by HMBC and NOE correlations (see Fig. 2). Likewise, analysis of the ^1H and ^{13}C NMR data and HMBC and NOE correlations led to establish the presence of a geranyl moiety in the alkoxycarbonylmethyl group located on carbon C-6 of the ring. Thus, the structure of

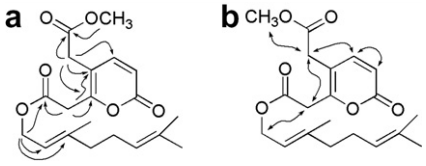


Fig. 2. Selected HMBC (a) and NOE (b) correlations of compound **2**.

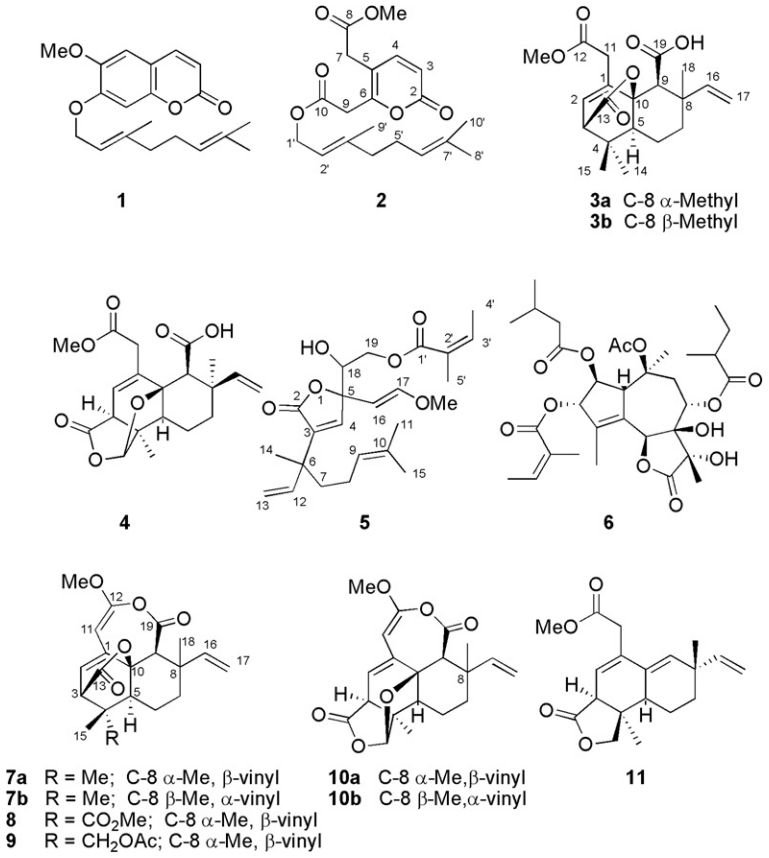


Fig. 1. Structure of compounds **1**–**11**.

2 was unambiguously elucidated as 6-geranyloxycarbonylmethyl-5-methoxycarbonylmethylpyran-2-one.

Compound **3a** gave the molecular formula $C_{20}H_{26}O_6$ as determined by HREIMS at *m/z* 362.1729, indicating eight degrees of unsaturation. The signals of the ^1H and ^{13}C NMR spectra suggested that the structure of compound **3a** was closely related to transtaganolide D (**7a**), being the main difference the lack of the signals attributed to the 7-methoxy-4,5-dihydro-3H-oxepin-2-one ring characteristic of transtaganolides A–D (Saouf et al., 2005). Instead, the presence of two coupled signals at δ_H 3.30 (H-11, dd) and 3.46 (H-11', dd) correlated in the HMBC spectrum with the carbons at δ_C 171.9 (C-12), 130.8 (C-2) and 137.8 (C-1), together with absorption bands in the IR spectrum typical of an ester group, indicated that a methoxycarbonylmethyl unit is situated at C-1. The presence of a carboxy group attached to C-9 was deduced from the IR spectrum (3450 and 1739 cm^{-1}) and HMBC correlations. These data are in accordance with the structure **3a** which can be formally derived from **7a** by opening of the oxepin-2-one ring. We named compound **3a** as transtaganolide E.

The molecular ion $C_{20}H_{26}O_6$determined for **3b** by HREIMS and the spectroscopic similarities, suggested that it might be an isomer of **3a**. On the basis of its ^1H–^1H COSY and HMBC spectra, together with the chemical shifts and coupling constants (Table 1), **3b** (transtaganolide F) was assumed to be the C-8 epimer of **3a**. In a similar way, **3b** can be derived from the ring opening of the oxepin-2-one moiety in **7b**.

Compound **4** showed a molecular ion at *m/z* 376.1532 which accounted for a $C_{20}H_{24}O_7$ and nine degrees of insaturation. An inspection of the ^1H NMR spectrum showed a structure closely related to that of transtaganolide A (**10a**), derived from the opening of the oxepin-2-one ring, a relation already described between **3a** and **3b** with **7a** and **7b** respectively. Thus, the structure of **4** (transtaganolide G) resulted to be that depicted in Fig. 1.

Compound **5** gave a molecular formula of $C_{24}H_{34}O_6$, as determined by HREIMS, at *m/z* 418.2335 $[M]^+$, representing to eight degrees of unsaturation. Its ^{13}C NMR showed the presence of two carbonyl groups and ten vinyl carbons, which accounted for seven degrees of unsaturation, indicating the monocyclic nature of the compound. The proton signal at δ_H 6.99 (H-4) was correlated in the HMBC spectrum with the carbon signals at δ_C 170.1, 140.4 and 86.5 which corresponded to carbons C-2, C-3 and C-5, respectively. This fact, together with an absortion band a 1747 cm^{-1} in the IR spectrum, suggested the presence of a furan-2-one ring. A detailed study of the ^1H–^1H COSY and HMBC correlations indicated that three different fragments were attached to the furan-2-one core. Thus, the ^1H NMR spectrum displayed the presence of a vinyl group, as could be deduced from the presence of a double doublet at δ_H 6.03 (*dd*, $J = 17.4$, 10.4 Hz) attributable to H-12 coupled with two signals at δ_H 5.11 and 5.05. The proton signal of H-12 was correlated in the HMBC spectrum with the carbons at δ_C 140.4 (C-3), 40.9 (C-6), 38.0 (C-7) and 21.9 (C-13). In addition, C-6 was attached at a 4-methylpent-3-enyl group, as was confirmed by HMBC and ^1H–^1H COSY correlations. Furthermore, the quaternary carbon C-6 was correlated in the HMBC spectrum with a singlet signal of a methyl group centered at δ_H 1.33. All the above information led us to propose the partial structure shown in Fig. 3.

The nature of the two remaining substituents attached to C-5 was established as follow. The ^1H NMR spectrum showed a deshielded doublet at δ_H 6.55 (H-17, *d*, $J = 13.3$ Hz) coupled to a signal at δ_H 5.01 (H-16, *d*, $J = 13.3$ Hz). The value of $J_{16,17}$ (13.3 Hz) was consistent with a *E* configuration of the double bond and the chemical shifts of proton H-16 and carbon C-16 (δ_C 98.4) suggested the presence of an electron-rich double bond. In the HMBC spectrum, the signal at δ_H 3.56 (3H, *s*) corresponding to a methoxy group was correlated with the carbons at δ_C 151.1 and 98.4 attributable to carbons C-17 and C-16, respectively. The attachement of the 2-methoxyethenyl group to C-5 was confirmed by HMBC correlations between H-16 and H-17 with carbons C-5 and C-4. Finally, the carbon C-5 also showed HMBC correlation with the signal at δ_H 3.92 (H-18) assignable to a proton geminal to a secondary hydroxyl group that was coupled to a isolated methylene as was confirmed by HMBC and ^1H–^1H COSY correlations. The chemical shift of the protons and the carbon of this methylene proved the presence of an ester group attached to C-19. The spectroscopic data of this ester were characteristic of an angelate group (Table 1). Assembling together all these fragments resulted the planar structure depicted in Fig. 1, that we named thapsianolide (5-(2-angeloyloxy-1-hydroxyethyl)-3-linaloyl-5-((*E*)-2-methoxyethenyl)-(5H)-furan-2-one).

We have not been able yet to determine the relative stereochemistry of thapsianolide (**5**), and furthermore, a detailed reinvestigation of the fraction where **5** was isolated, revealed the presence of at least two minor isomers of this compound in amounts not sufficient to be fully characterized.

As transtaganolides could formally be considered as norditerpenes, Appendino et al. proposed a mevalonic-based biosynthetic pathway to explain the biogenesis of these compounds (Appendino et al., 2005). In the same publication, these authors suggested a very different proposal which consider transtaganolides as meroterpenoids derived from a C-prenylated coumarin **12** via a tandem oxidation (to **13**) – electrocyclic ring opening (to **14**) – Diels-Alder cycloaddition (Scheme 1).

The characterization of both pyran-2-one **2** and 7-*O*-geranylscopoletin (**1**) (also isolated from other plants belonging to the genus *Thapsia* (Larsen and Sandberg, 1970; Rasmusssen et al., 1981)), as metabolites of *T. transtagana* permits to propose others possible biosynthetic pathways to transtaganolides and supports their meroterpenoid origin. Direct oxidation of **1** to **15**, followed of an electrocyclic ring opening to **16** and further hydrolytic cleavage yield **2.**

Table 1
NMR data of **2**, **5**, **3a**, **3b** and **4**

2[a]			**5**[a]			**3a**[a]			**3b**[b]		**4**[a]	
	δ_H (int, mult. *J*)	δ_C		δ_H (int, mult. *J*)	δ_C		δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C	δ_H (int, mult. *J*)	δ_C
2	–	161.5	**2**	–	170.1	**1**	–	137.8	–	138.2	–	144.8
3	6.24 (1H, *d*, 9.5)	115.3	**3**	–	140.4	**2**	6.22 (1H, *bd*, 6.5)	130.8	5.71 (1H, *bd*, 6.5)	130.5	5.69 (1H, *d*, 6.0)	126.1
4	7.25 (1H, *d*, 9.5)	146.6	**4**	6.99 (1H, *d*, 1.1)	147.7	**3**	2.94 (1H, *d*, 6.5)	53.9	2.67 (1H, *d*, 6.5)	53.7	3.05 (1H, *d*, 6.0)	51.6
5	–	111.2	**5**	–	86.5	**4**	–	36.6	–	36.2	–	49.4
6	–	155.7	**6**	–	40.9	**5**	1.65 (1H, *m*)	48.3	1.50 (1H, *dd*, 12.9, 4.4)	48.1	2.23 (1H, *dd*, 11.8, 6.3)	48.5
7	3.33 (2H, *s*)	34.9	**7**	1.66 (1H, *m*)	37.9	**6**	1.58 (2H, *m*)	20.9	1.12 (1H, *m*)	20.6	1.47 (1H, *m*)	20.5
8	–	170.4		1.83 (1H, *m*)					1.40 (1H, *m*)		1.65 (1H, *m*)	
9	3.57 (2H, *s*)	37.7	**8**	1.81 (2H, *m*)	23.1	**7**	1.60 (1H, *m*)	38.9	1.47 (1H, *m*)	38.7	1.48 (1H, *m*)	37.9
10	–	167.7	**9**	5.06 (1H, *bt*, 7.1)	123.9		1.88 (1H, *d*, 11.2)		1.30 (1H, *m*)		1.87 (1H, *m*)	
1′	4.62 (2H, *bd*, 7.2)	62.9	**10**	–	131.8	**8**	–	39.9	–	39.9	–	39.3
2′	5.29 (1H, *bt*, 7.2)	117.6	**11**	1.64 (3H, *s*)	25.6	**9**	3.00 (1H, *s*)	54.8	3.18 (1H, *s*)	55.5	2.88 (1H, *s*)	56.2
3′	–	143.5	**12**	6.03 (1H, *dd*, 17.5, 10.7)	142.7	**10**	–	84.4	–	83.7	–	85.5
4′	2.05 (2H, *m*)	39.4	**13**	5.05 (1H, *dd*, 17.5, 0.7)	113.6	**11**	3.30 (1H, *dd*, 17.2, 1.3)	36.8	3.24 (1H, *dd*, 17.4, 1.1)	38.8	3.20 (1H, *d*, 18.2)	38.8
5′	2.10 (2H, *m*)	26.4		5.11 (1H, *dd*, 10.7, 0.7)			3.46 (1H, *dd*, 17.2, 1.3)		3.72 (1h, *dd*, 17.4, 1.1)		3.65 (1H, *d*, 18,2)	
6′	5.05 (1H, *bt*, 7.2)	123.8	**14**	1.33 (1H, *s*)	21.9	**12**	–	171.9	–	171.7	–	173.0
7′	–	132.1	**15**	1.56 (3H, *s*)	17.6	**13**	–	172.5	–	171.3	–	175.9
8′	1.66 (3H, *s*)	25.9	**16**	5.01(1H, *d*, 13)	98.4	**14**	0.97 (3H, *s*)	29.7	0.68 (3H, *s*)	29.2	1.28 (3H, *s*)	15.9
9′	1.57 (3H, *s*)	17.9	**17**	6.55 (1H, *d*, 13)	151.1	**15**	1.04 (3H, *s*)	25.2	0.85 (3H, *s*)	24.9	5.59 (1H, *s*)	110.0
10′	1.67 (3H, *s*)	16.7	**18**	3.92 (1 H, *dd*, 7.0, 3.4)	74.3	**16**	6.57 (1H, *dd*, 17.4, 11.0)	141.3	6.00 (1H, *dd*, 17.3, 10.7)	147.9	6.57 (1H, *dd*, 17.7, 11.1)	141.3
MeO	3.70 (3H, *s*)	52.7	**19**	4.20 (1 H, *dd*, 11.9, 7.0)	64.4	**17**	5.11 (1H, *dd*, 11.0, 1.0)	113.6	5.04 (1H, *dd*, 10.7, 0.7)	112.6	5.05 (1H, *dd*, 11.1, 1.0)	113.1
				4.29 (1 H, *dd*, 11.9, 3.4)			5.05 (1H, *dd*, 17.4, 1.0)		5.14 (1H, *dd*, 17.3, 0.7)		5.00 (1H, *dd*, 17.7, 1.0)	
			1′	–	168.1	**18**	1.30 (3H, *s*)	29.4	1.57 (3H, *s*)	18.8	1.22 (3H, *s*)	29.3
			2′	–	127.1	**19**	–	166.4	–	167.1	–	167.1
			3′	6.17 (1H, *bq*, 6.8)	140.0	**MeO**	3.70	52.4	3.27 (3H, *s*)	51.4	3.71 (3H, *s*)	52.4
			4′	1.98 (3H, *dq*, 7.4, 0.8)	15.9							
			5′	1.89 (3H, *bs*)	20.5							
			MeO	3.56 (3H, *s*)	56.3							
			OH	2.66								

[a] Inova 400 MHz, $CDCl_3$.
[b] Inova 600 MHz, C_6D_6.

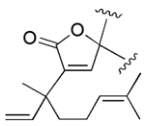


Fig. 3. Partial structure of compound **5**.

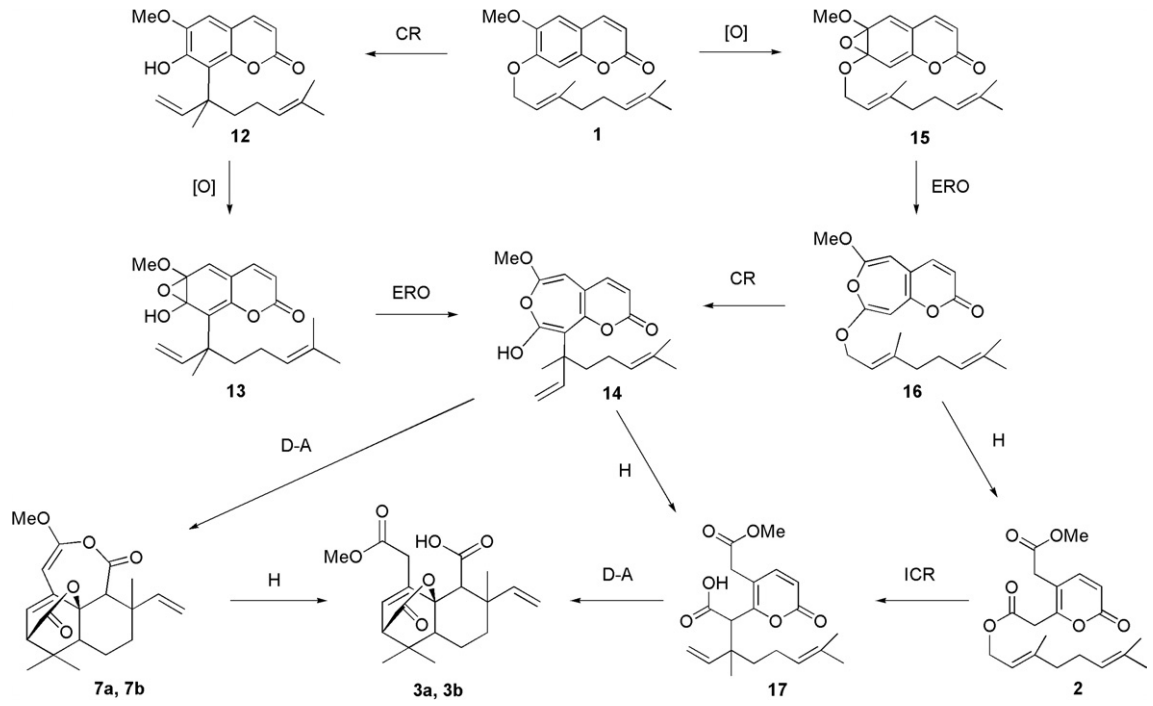
Likewise, **14** could be obtained from **16** via a Claisen rearrangement instead of the route proposed by Appendino et al. (2005) starting from **12**. The occurrence of C-8 epimeric transtaganolides can be explained taking into account that the Claisen rearrangement proceeds without diastereoselectivity. Despite *ortho*-dialkoxy aryl fragmentation (Schmidt et al., 1989) is least frequent than phenol fragmentation (Vaillancourt et al., 2006) the presence of pyrone **2** (that can be considered a seco-coumarin) supports the direct fragmentation of **1**.

A subsequent Ireland-Claisen rearrangement of **2** to **17** followed by a Diels-Alder cycloaddition would provide the transtaganolide derivatives **3a** and **3b**. Alternatively, **17** could be obtained by a hydrolysis of **14**, and in a similar way **7a** and **7b** yield **3a** and **3b** (this transformation has been already observed by us in NMR samples of **7a** in $CDCl_3$ on standing).

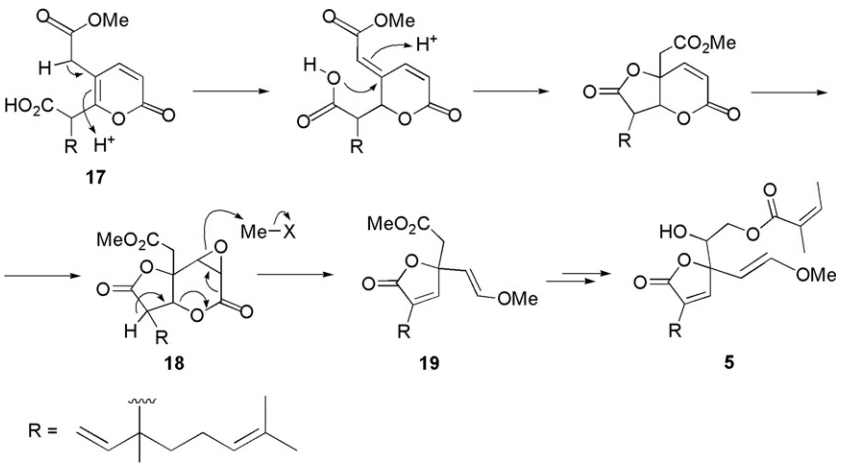
The members of the transtaganolide family, **8** and **9** (Appendino et al., 2005), can be considered as derivatives of **7a** and **7b** through oxidation at C-14. In the case of transtaganolides C and D (**10a**, **10b)** (Saouf et al., 2005), the aldehyde resulting from the oxidation at C-15 is trapped as lactone ketal by the hydroxyacid formed after the opening of the C-10/C-3 lactone. Haplonolide (**11**) (Gunes, 2001) should be derived from **3b** by oxidation at C-15, opening of the C-10/C-3 lactone, relactonization to form the C-13/C-15 lactone and decarboxylation of the corresponding β-hydroxyacid. It is noteworthy that the sterochemical pattern of transtaganolides comes from only one of the four ways to approach of the Diels-Alder cycloaddition.

To the best of our knowledge this cascade reaction for biosynthetic construction of complex molecules has a unique precedent in a growing class of biologically active natural products isolated from the genus *Garcinia* over 36 years ago (Quillinan and Scheinmann, 1971). To explain the origin of this class of secondary metabolites, Quillinan and Scheinmann postulated a Claisen rearrangement followed by an intramolecular D-A reaction. These authors succeded in synthesizing these structures by means of such electrocyclic process. Subsequently, other biomimetic syntheses have been described (Nicolaou and Li, 2001; Tislade et al., 2004).

Finally, intermediate **17** also can be presumed a precursor of the unusual branched γ-lactone **5**. The proposal involves isomerization of the C-5/C-6 double bond, lactonization, and epoxidation of the C-3/C-4 double bond to afford the epoxydilactone **18** (Scheme 2). Methylation of the epoxide and decarboxylation give rise the lactone **19** which bears two of the three appendices present in **5.** The



Scheme 1. Biogenetic proposals for transtaganolides (CR: Claisen rearrangement, ERO: electrocyclic ring opening, D-A: Diels-Alder cycloadition, ICR: Ireland-Claisen rearrangement, H: hydrolysis).



Scheme 2. Proposed biogenesis of lactone **5**.

remaining C_2 unit, the methoxycarbonylmethyl substituent, should afford the corresponding 1-hydroxy-2-angeloyloxy moeity.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin Elmer Model 341 digital polarimeter. IR spectra were measured in a Perkin Elmer Spectrum BX spectrophotometer.^{1}H (1D, DQF-COSY, 1D-NOESY, 2D-NOESY) and ^{13}C (1D, gHSQC, HMBC) NMR spectra were recorded in Varian Inova 400 and Inova 600 spectrometers. Mass spectra were recorded at the Mass Spectra Facilities of Universidad de Alicante (Spain). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on plates precoated with silica gel F254 (Merck, Germany).

3.2. Plant material

Specimens of *T. transtagana* were collected near Bouznika (Morocco) and a voucher of the plant is deposited in the Departamento de Ciencias y Recursos Agrícolas y Forestales of the University of Córdoba (Spain).

3.3. Isolation

The roots of *T. transtagana* (900 g) were grounded and extracted with dichloromethane in a Soxhlet apparatus, yielding 13 g of an oily residue, which was purified by column chromatography with increasing polarities of EtOAc/hexanes mixtures. The 1:3 and 1:4 EtOAc/hexane-eluted fractions yielded after further purification by column chromatography compounds **1** (20 mg), **2** (9 mg), **3a** (6.2 mg), **3b** (7.6 mg), **4** (20 mg) and **5** (3 mg).

3.3.1. Compound **2**

Colourless oil; IR (NaCl) ν_{max} cm^{-1}: 2926, 1740, 1648, 1557, 1237, 1164, 1102, 828; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m/z* 362.1752 (calcd. for $C_{20}H_{26}O_6$, 362.1729); EIMS 70 eV, *m/z* (rel. int.): 227 (14), 182 (15), 135 (27), 95 (38), 82 (65), 55 (45), 43 (100).

3.3.2. Compound **3a**

Yellow oil; $[\alpha]_D^{20}$ + 10.7° (*c* 0.10, $CHCl_3$); IR (NaCl) ν_{max} cm^{-1}: 3446, 2964, 1812, 1738, 1172, 1034, 1002, 756; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m/z* 362.1732 (calcd. for $C_{20}H_{26}O_6$, 362.1729); EIMS 70 eV, *m/z* (rel. int.): 313 (100), 300 (79), 285 (65), 272 (69), 199 (53), 197 (63).

3.3.3. Compound **3b**

Yellow oil; $[\alpha]_D^{20}$ + 4.0° (*c* 0.47, $CHCl_3$); IR (NaCl) ν_{max} cm^{-1}: 3440, 2966, 2855, 1809, 1746, 1339, 1249, 1169, 978, 754; ^{1}H NMR (C_6D_6, 600 MHz) and ^{13}C NMR (C_6D_6, 150 MHz) data, see Table 1; HREIMS *m/z* 345.1699 $[M–OH]^+$ (calcd. for $C_{20}H_{25}O_5$, 345.1702); EIMS 70 eV, *m/z* (rel. int.): 345 (15), 301 (35), 273 (100), 199 (40).

3.3.4. Compound **4**

Yellow oil; $[\alpha]_D^{20}$ − 33.3° (*c* 0.10, $CHCl_3$); IR (NaCl) ν_{max} cm^{-1}: 3449, 3011, 1760, 1185, 758; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m/z* 376.1532 (calcd. for $C_{20}H_{24}O_7$, 376.1522); EIMS 70 eV, *m/z* (rel. int.): 358 (41), 327 (99), 257 (42), 225 (52), 197 (100).

3.3.5. Compound **5**

Yellow oil; $[\alpha]_D^{20}$ − 4.8° (*c* 0.10, $CHCl_3$); IR (NaCl) ν_{max} cm^{-1}: 3447, 2926, 2864, 1747, 1650, 1454, 1378, 1229, 1151; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m/z* 418.2335 (calcd. for $C_{24}H_{34}O_6$, 418.2355); EIMS 70 eV, *m/z* (rel. int.): 300 (23), 242 (24), 241 (100), 225 (59), 197 (63).

Acknowledgements

The authors are grateful to the Consejería de Medio Ambiente (Junta de Andalucía) for financial support.

References

Appendino, G., Prosperini, S., Valdivia, C., Ballero, M., Colombano, G., Billington, R.A., Genazzani, A.A., Sterner, O., 2005. SERCA-Inhibiting activity of C-19 terpenolides from *Thapsia garganica* and their posible biogenesis. J. Nat. Prod. 68, 1213–1217.

Breda, S., Lapinski, L., Fausto, R., Nowak, M.J., 2003. Photoisomerization reactions of 4-methoxy- and 4-hydroxy-6-methyl-α-pyrones: An experimental matrix isolation and theoretical density funtional theory study. Phys. Chem. Chem. Phys. 5, 4527–4532.

Christensen, S.B., Andersen, A., Smitt, U.W., 1997. Sesquiterpenoids from *Thapsia* species and medicinal chemistry of the thapsigargins. Prog. Chem. Org. Nat. Prod. 71, 129–167.

Furuya, Y., Lundmo, P., Short, A.D., Gill, D.L., Isaac, J.T., 1994. The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. Cancer Res. 54, 6167–6175.

Gunes, H.S., 2001. (−)-Haplonolide, a novel terpenoid from *Haplophyllum vulcanicum*. J. Fac. Pharm. Gazi. 18, 35–41.

Larsen, P.K., Sandberg, F., 1970. Constituents of Umbelliferous plants. XV. Coumarins from *Thapsia garganica* L. The structure of a new coumarin. Acta Chem. Scand. 24, 1113–1114.

Liu, H., Jensen, K.G., Tran, L.M., Chen, M., Zhai, L., Olsen, C.E., Søhoel, H., Denmeade, S.R., Isaacs, J.T., Christensen, S.B., 2006. Cytotoxic phenylpropanoids and an additional thapsigargin analogue isolated from *Thapsia garganica*. Phytochemistry 67, 2651–2658.

Navarrete, C., Sancho, R., Caballero, F.J., Pollastro, F., Fiebich, B.L., Sterner, O., Appendino, G., Muñoz, E., 2006. Basiliolides, a class of tetracyclic C-19 dilactones from *Thapsia garganica*, release Ca^{2+} from the endoplasmatic reticulum and regulate the activity of the transcription factors of activated T cells, nuclear factor-kB and activator protein 1 in T lymphocytes. J. Pharm. Exp. Therap. 319, 422–430.

Nicolaou, K.C., Li, J., 2001. Biomimetic cascade reactions in organic synthesis: construction of 4-oxatricyclo-[4.3.1.0]decan-2-one systems and total synthesis of 1-*O*-methylforbesione via tandem Claisen rearrangement/Diels-Alder reactions. Angew. Chem. Int. Ed. 40, 4264–4268.

Pujadas, J.A., Roselló, J.A., 2003. *Thapsia* L. In: Nieto Feliner, G., Jury, S.L., Herrero, A. (Eds.), Flora Ibérica, vol. 10. Real Jardín Botánico, CSIC, Madrid, pp. 401–410.

Quillinan, A., Scheinmann, F., 1971. Application of the rearrangement to the synthesis of biciclo[2,2,2]octenones: an approach to the morellins based on new biogenetic suggestions. Chem. Commun., 966–967.

Rassmussen, U., Christensen, S.B., Sandberg, F., 1978. Thapsigargin and thapsigargicin, two new histamine liberators from *Thapsia garganica* L. Acta Pharm. Suec. 15, 133–140.

Rasmusssen, U., Christensen, S.B., Sandberg, F., 1981. Phytochemistry of the genus *Thapsia*. Planta Med. 43, 336–341.

Saouf, A., Guerra, F.M., Rubal, J.J., Moreno-Dorado, F.J., Akssira, M., Mellouki, F., López, M., Pujadas, A.J., Jorge, Z.D., Massanet, G.M., 2005. Transtaganolides A–D: novel metabolites from *Thapsia transtagana*. Org. Lett. 7, 881–884.

Saouf, A., Guerra, F.M., Rubal, J.J., Jorge, Z.D., Akssira, M., Mellouki, F., Moreno-Dorado, F.J., Massanet, G.M., 2006. Phenylpropanoids from *Thapsia transtagana*. Phytochemistry 67, 800–804.

Schmidt, H.W.H., Haemmerli, S.D., Schoemaker, H.E., Leisola, M.S.A., 1989. Oxidative degradation of 3,4-dimethoxybenzyl alcohol and its methyl ether by the lignin peroxidase of *Phanerochaete chrysosporium*. Biochemistry 28, 1776–1783.

Tislade, E.J., Slobodov, I., Theodorakis, E.A., 2004. Unified synthesis of caged Garcinia natural products based on site-selectiv Claisen/Diels-Alder/Claisen rearrangement. Proc. Nat. Acad. Sci. 101, 12030–12035.

Treiman, M., Caspersen, S.B., Christensen, S.B., 1998. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends Pharmacol. Sci. 19, 131–136.

Vaillancourt, F.H., Bolin, J.T., Eltis, L.D., 2006. The ins and outs of ring-cleaving dioxigenases. Crit. Rev. Biochem. Mol. Biol. 41, 241–267.